

# Impaired CD4<sup>+</sup>CD69<sup>+</sup> cells activation associated with elevated IL-6 and IL-10 in Togolese SLE patients

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**ABSTRACT**

**Introduction:** Systemic lupus erythematosus (SLE) is an autoimmune disease involving complex pathogenesis which remains poorly investigated in developing countries. Indeed, mononuclear blood cells and cytokines play key role in the pathogenesis of the disease. The aim of this study was to investigate the mononuclear blood cells and their cytokines profile in togolese SLE patients. **Methods:** A cross-sectional study was performed on five SLE patients and seven healthy donors. Mononuclear blood cells were characterized by flow cytometry. Cytokines from plasma: IL-6, TNF $\alpha$ , IFN $\gamma$ , IL-5, IL-10 and IL-17A were assayed by sandwich ELISA. **Results:** CD4<sup>+</sup>cells count was decreased with an impaired activation showed by low expression of activation marker CD69 in SLE patients compared to healthy donors. SLE patients were also characterized by high production of plasma IL-6 and IL-10. **Conclusion:** Togolese SLE patients are characterized by impaired CD4<sup>+</sup>CD69<sup>+</sup> cells activation, associated with high plasma levels of IL-6 and IL-10.

**Keywords:** Systemic lupus erythematosus, mononuclear blood cells, cytokines, SLE patients, Togo.

**1. INTRODUCTION**

Systemic lupus erythematosus (SLE) is a non-organ-specific autoimmune disease characterized by high production and anti-nuclear antibodies (ANAs) such as anti-DNA, anti-RNA, anti-histone, anti-Sm, anti-U1-RNP, anti-SSA, anti-SSB antibodies; and a clinical heterogeneity Tsokos, (2011). It affects thousands of people worldwide with a sex ratio of nine women for one-man Ramírez Sepúlveda et al., (2019) and has been shown to induce severe outcomes in african origin people Ocampo-Piraquive et al., (2018).

The pathophysiology of SLE is complex involving mononuclear blood cells and cytokines Möckel et al., (2021). These mononuclear blood cells play an

important role in the body's defense against many aggressors and their role in autoimmune diseases such as SLE have been widely investigated (Mortezaghali et al., 2016; Dar et al., 2012). Mediation between different cells is maintained by cytokines that they secrete Raphael et al., (2015). Figure 1 SLE pathogenesis overview. Several factors are incriminated in the complexity of SLE pathogenesis; namely genetic, hormonal, environmental and immuno-regulatory factors. Following exposure to UV radiation, high apoptosis of keratinocytes occurs, releasing auto-antigens consisting of RNA, DNA and apoptotic fragments which are captured by dendritic cells (DC) and presented to CD4<sup>+</sup>T cells, inducing the production of auto-antibodies by collaborating with B cells. Resulting immune complexes precipitate in tissues and activate the complement which attracts phagocytic cells. The latter, with CD4<sup>+</sup>T cells, produce inflammatory cytokines such as IL-6, TNF $\alpha$ , IFN $\gamma$ , IL-17A contributing to the necrosis of the target tissues (skin, kidneys, lungs, brain and heart).

In Sub-Saharan Africa, several studies have investigated the prevalence of anti-nuclear antibodies, anti-phospholipid antibodies, treatments and clinical symptoms of SLE (Kombate et al., 2008; Zomalheto et al., 2014). But few have investigated the role of mononuclear blood cells and their cytokines during SLE. Since environmental and genetic factors influence the pathogenesis of SLE, this study aimed to investigate the mononuclear blood cells and their cytokines profile in Togolese SLE patients of CHU Sylvanus Olympio (Sylvanus Olympio University Hospital) in Lomé.

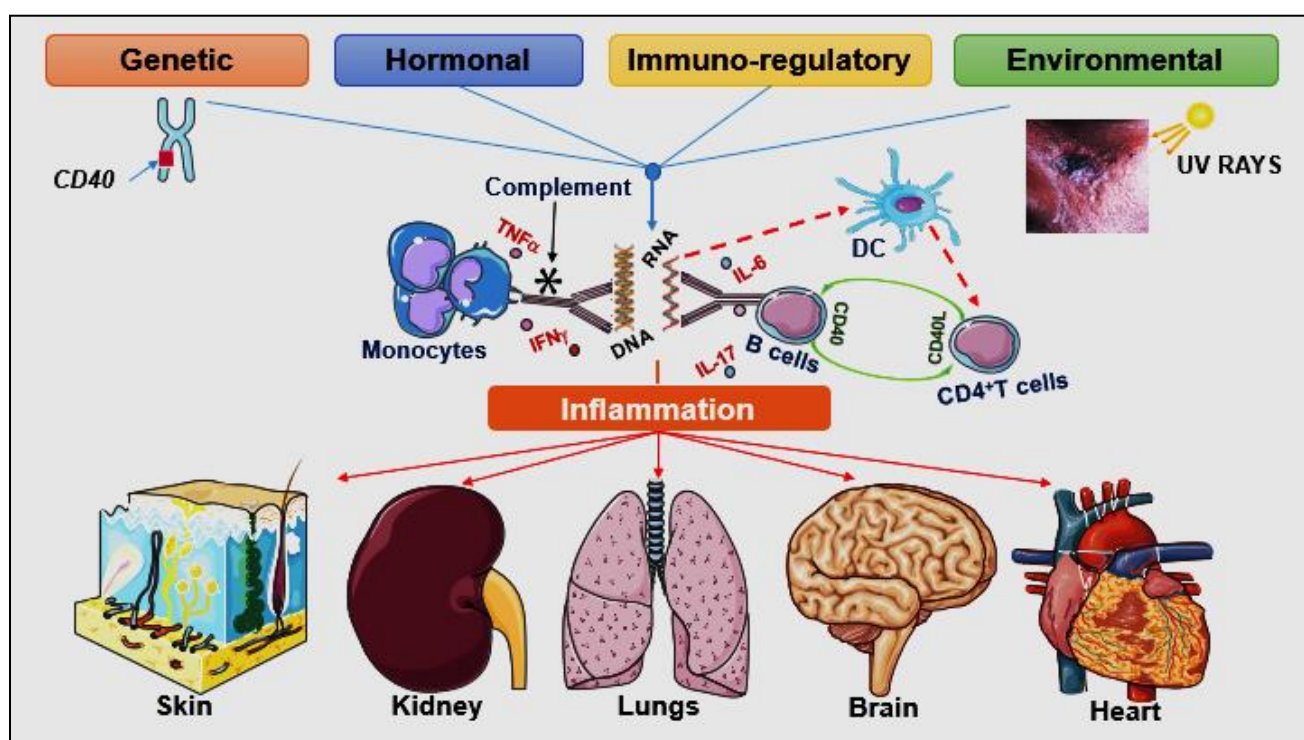


Figure 1 SLE pathogenesis overview

## 2. METHODS

### Study population and area

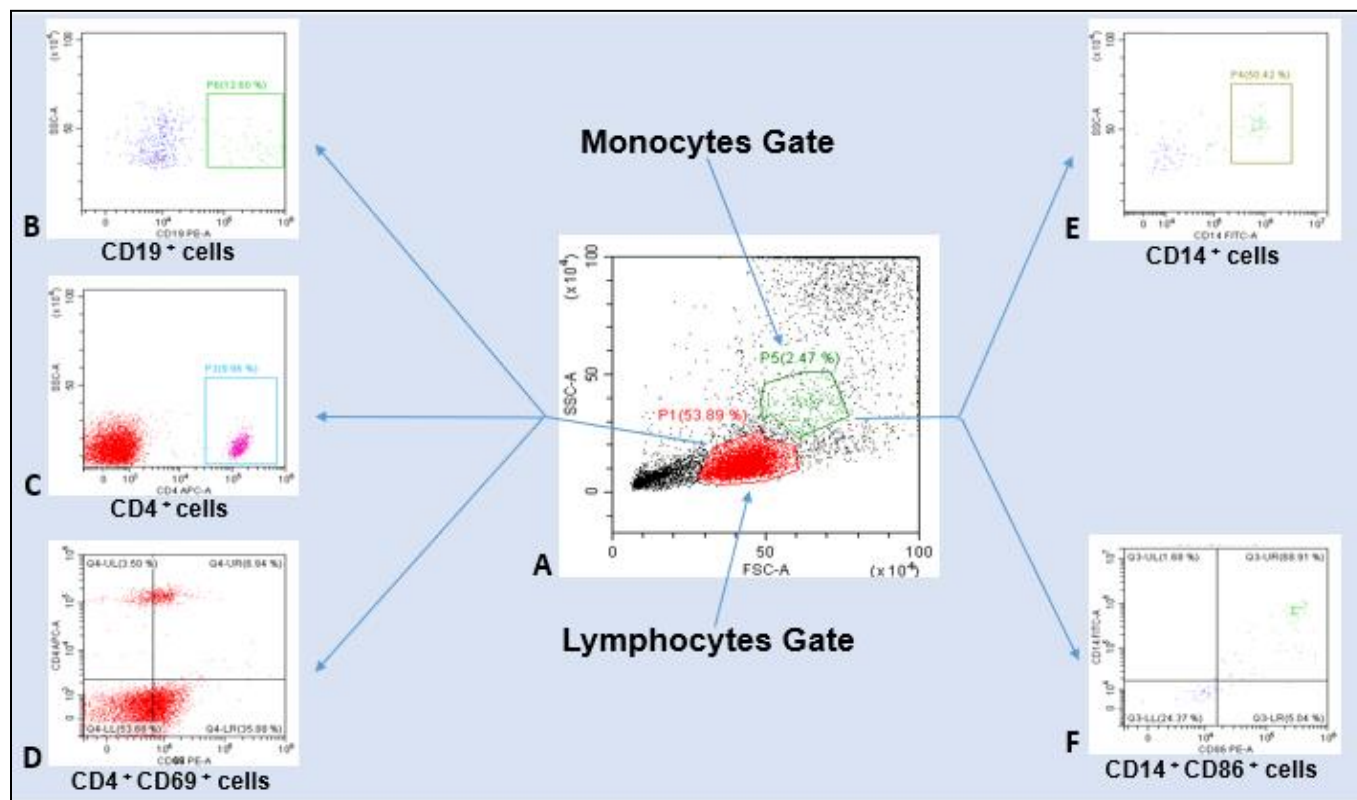
Five consecutive togolese SLE patients were enrolled in a cross-sectional study from March to June 2019, at the rheumatology and dermatology departments of CHU Sylvanus Olympio (University hospital center in Lomé). Also, seven healthy donors from the national blood transfusion center of Lomé (CNTS), were included in this study as control group. Demographic informations and ongoing treatment were acquired for SLE patients at time of study inclusion. Anti-ssDNA antibodies assay, PBMCs isolation, cell culture, immuno-phenotyping and cytokines assay were done at the Research Unit in Immunology and Immuno-diagnosis: The clinical diagnosis of SLE was done by the physician, based on 1997 update of the 1982 ACR clinical criteria for classification of SLE which are: malar rash, discoid rash, photosensitivity, oral ulcers, nonerosive arthritis, pleuritis or pericarditis (pleuritic pain). Biological diagnosis was done on SLE patients' plasma, using Human single stranded DNA antibody ELISA kit (CUSABIO, Wuhan, China) according to manufacturer's instructions.

### Peripheral Blood Mononuclear Cells (PBMCs) isolation

SLE patients and healthy donors PBMCs were isolated by ficoll density gradient centrifugation. In brief, 20 mL of whole blood collected on EDTA were diluted with 15 mL of DPBS (Gibco, California, USA) and gently poured on 15 mL of ficoll (PAN Biotech, Aidenbach, Germany) in a 50mL falcon tube (Greiner, Kremsmünster, Austria) and centrifuged at 2000 rpm for 20 minutes. The white interphase of PBMCs was then recovered and washed twice by centrifugation at 1300 rpm for 8 minutes with supplemented RPMI (RPMI 1640 + gentamycin 50 µg/mL + Penicillin/Streptomycin 100 µg/mL + L-glutamine 2 mM/mL). The washed PBMCs were resuspended in 1mL of supplemented RPMI 1640 (Gibco, California, USA) with 10% of FBS (PAN Biotech, Aidenbach, Germany), and then their viability was checked by Trypan blue (Life Technologies Corporation, Grand Island, USA) exclusion technique. To this end, 1/100 dilution of suspended PBMCs was done with 0.4% Trypan Blue and counted using the Neubauer hemocytometer (Paul Marienfeld GmbH & Co. KG, Lauda-Königshofen, Germany). The estimation of the number of cells per mL was done according to the formula: Number of cells counted  $\times$  dilution factor  $\times 10^4$ . Immuno-phenotyping was performed when viability was above 50%.

### Immuno-phenotyping

PBMCs were stained for T helper cells, B cells and monocytes surface markers. Briefly, 100 µL containing  $10^5$  of fresh isolated PBMCs were washed by centrifugation at 1500 rpm for 5 minutes with 200 µL of FACS buffer (DPBS 2% FBS) and stained in dark with 1µL of anti-hCD4-APC (clone A161A1, BioLegend, Koblenz, Germany), anti-hCD14-FITC (clone 63D3, BioLegend, Koblenz, Germany), anti-hCD19-PE (clone HIB19, BioLegend, Koblenz, Germany), anti-hCD86-PE (clone IT2.2, BioLegend, Koblenz, Germany) and anti-hCD69-PE (clone FN50, BioLegend, Koblenz, Germany) monoclonal antibodies. The stained cells were incubated in dark for 30 min at 4°C and then washed as before and resuspended with 100 µL of FACS buffer for acquisition on flow cytometer (Beckman Coulter Technology, Suzhou, China). The fluorescence compensation was performed with VersaComp Antibody Capture Beads (Beckman Coulter, Brea, USA) and data were analyzed using CytExpert 2.1 software (Beckman Coulter Technology, Suzhou, China) according to the gating strategy (Figure 2).



**Figure 2** Gating strategy

A classic gating strategy was done (A); CD19<sup>+</sup> cells (B), CD4<sup>+</sup> T cells (C) and CD4<sup>+</sup>CD69<sup>+</sup> cells (D) were analyzed on lymphocytes gate; CD14<sup>+</sup> cells (E) and CD14<sup>+</sup>CD86<sup>+</sup> (F) were analyzed on monocytes gate.

### Cytokine assay

Cytokines such as TNF $\alpha$ , IL-6, IFN $\gamma$ , IL-5, IL-10, IL-17A were assayed from SLE patients and healthy donors plasma by sandwich ELISA using Invitrogen cytokines ELISA Kits (Thermo Fisher Scientific, Bender MedSystems GmbH, Vienna, Austria) according to the manufacturer's instructions. Then, systemic inflammatory balance was determined by a ratio of the highest systemic cytokines in SLE patients, according to the formula: (Mean of anti-inflammatory cytokine) / (Mean of pro-inflammatory cytokine)

### Statistical Analysis

Our data were analyzed with GraphPad PRISM 5.02 software for Windows (GraphPad Software, San Diego California USA). D'Agostino and Pearson omnibus normality test was performed to check the distribution of values. Since variables were non-parametric, Mann-Whitney U-test was performed to compare medians. Differences were considered significant for a p-value below 0.05.

## 3. RESULTS

### Characteristics of the study population

Table 1 presents the characteristics of the study population. The mean age of SLE patients was 32.20 years and all patients enrolled in this study were female. Healthy donors were also female with a mean age of 27.14 years. The mean duration of illness was 6.5 years for SLE patients. All of them were photosensitive whereas 60% had arthritis and 40%, had discoid rash and oral/nasopharyngeal ulcers. Anti-DNA antibodies were found in their plasma at a dilution of 1/101. They were treated with one or a combination of following drugs: 20% with Prednisone, 20% with Methotrexate and Prednisone, 20% with Azathioprine and Prednisone, 20% with Clobetasol propionate cream and 20% with Locoid ointment, Mequitazine and Fucidic acid ointment (Table 1).

**Table 1** Demographic, clinical and biological data of the study population

Variables	SLE patients	Healthy donors
Gender		
Female n (%)	5 (100)	7 (100)
Age (years)	32.2	27.14
Duration of illness (years)	6.5	NA
Clinical and biological outcomes		
Discoid rash (%)	40	NA
Photosensitivity (%)	100	NA
Oral/nasopharyngeal ulcers (%)	40	NA
Arthritis (%)	60	NA
Anti-DNA Ab (%)	100	NA
Treatments		
Prednisone n (%)	1 (20)	NA
Methotrexate and Prednisone n (%)	1 (20)	NA
Azathioprine and Prednisone n (%)	1 (20)	NA
Clobetasol propionate cream n (%)	1 (20)	NA
Locoid ointment, Mequitazine and Fucidic acid ointment n (%)	1 (20)	NA

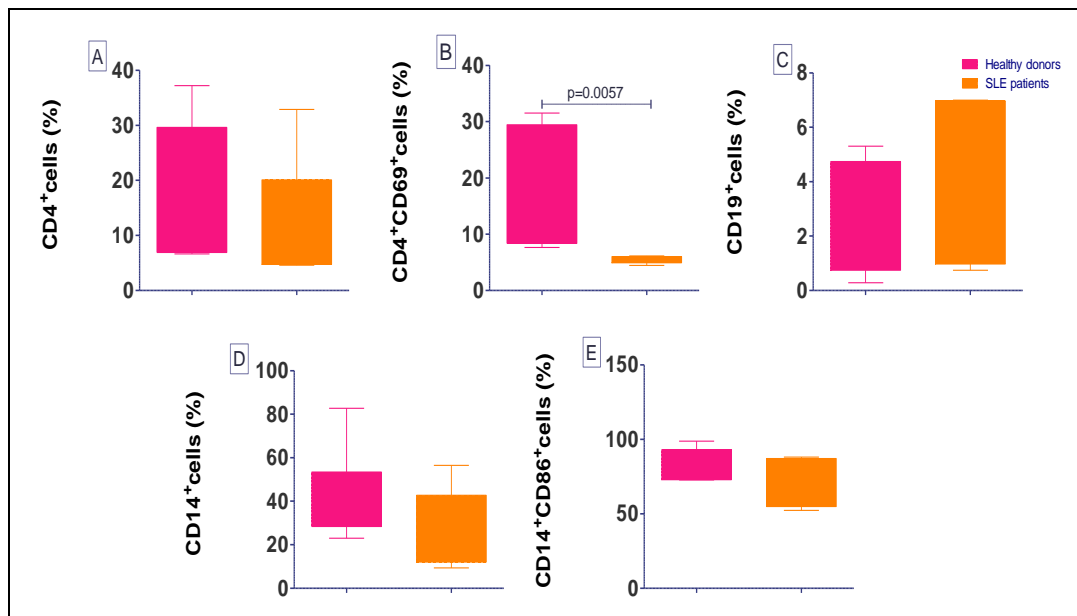
NA=Not applicable

Table 1 shows the gender, mean age, duration of illness, clinical outcomes, immunological marker and ongoing treatments of SLE patients and/or healthy donors.

### Mononuclear blood cells profile

Low expression of CD4<sup>+</sup> T cells and CD14<sup>+</sup> cells were observed in SLE patients. There was a significant defect of activation of SLE patients CD4<sup>+</sup> T cells showed by a low frequency of CD4<sup>+</sup> T population expressing CD69 marker. In contrast SLE patients had high expression of CD19<sup>+</sup> B cells compared to healthy donors (Figure 3).

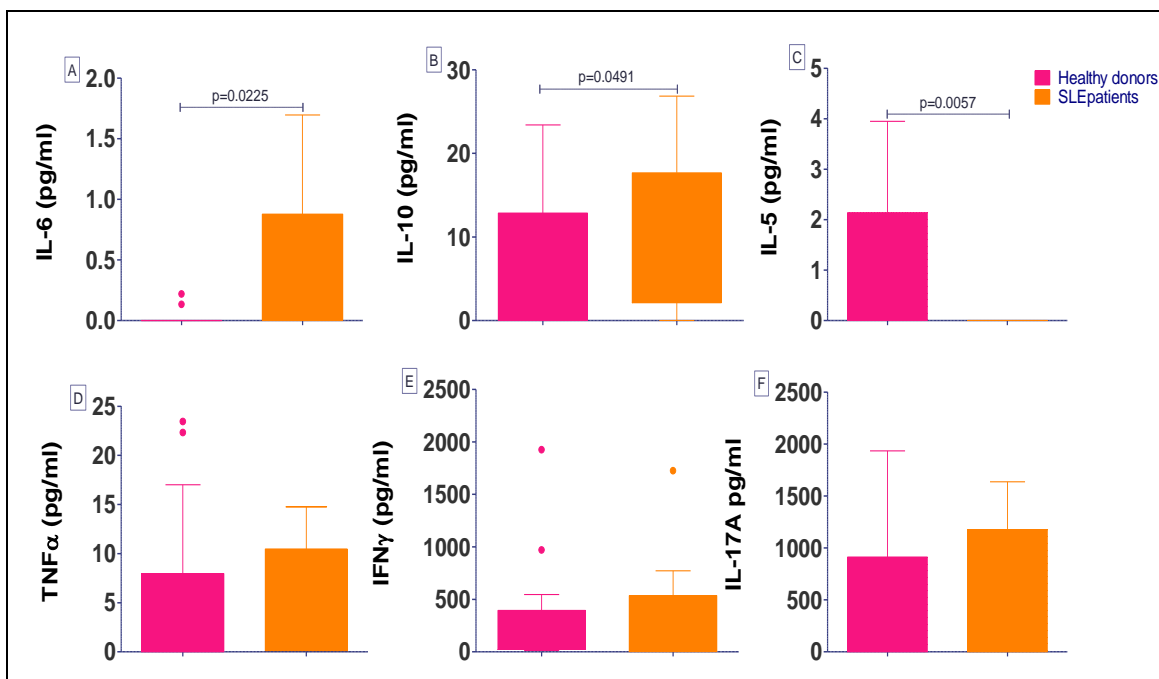
Box whiskers (tukey) with outliers show the percentages of CD4<sup>+</sup>cells (A), CD4<sup>+</sup>CD69<sup>+</sup>cells (B), CD19<sup>+</sup>cells (C), CD14<sup>+</sup>cells (D) and CD14<sup>+</sup>CD86<sup>+</sup>cells (E) in SLE patients compared to healthy donors. p values were determined by Mann-Whitney U-test.



**Figure 3** Mononuclear blood cells profile

### Cytokines profile

Systemic cytokines assay showed that SLE patients had an inflammatory environment characterized by significant production of IL-6 ( $p = 0.0225$ ). At the same time, their inflammatory milieu was balanced by significant level of IL-10 ( $p = 0.0491$ ), when compared to healthy donors. But unexpectedly, IL-5 level was undetectable in these patients (Figure 4).



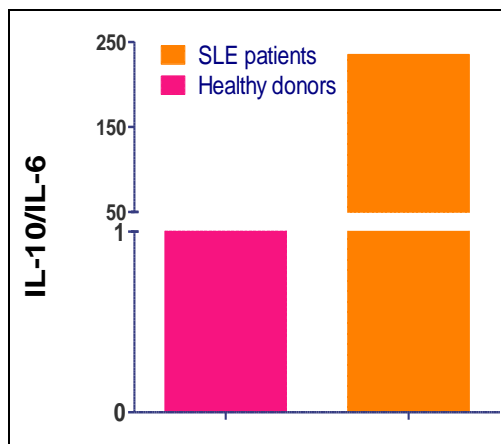
**Figure 4** Plasmatic cytokines profile

Box whiskers (tukey) with outliers show the concentrations of IL-6 (A), IL-10 (B), IL-5 (C), TNF $\alpha$  (D), IFN $\gamma$  (E) and IL-17A (F) in SLE patients and healthy donors plasma.  $p$  values were determined by Mann-Whitney U-test.

### Inflammatory balance

As SLE patients had significant plasmatic level of IL-6 and IL-10, we established a ratio to assess inflammatory balance in these patients. It appeared that as SLE patients were undertaken a treatment, their immune system was well regulated, showed by a ratio

above 1. Nevertheless, healthy donors had greater level of IL-10 than SLE patients (Figure 5). Figure 5 Inflammatory balance. Bars show IL-10/IL-6 ratio in SLE patients and healthy donors.



**Figure 5** Inflammatory balance

#### 4. DISCUSSION

This study was conducted on few SLE patients as the prevalence of SLE in Togo is low (40 cases consulted in 19 years) Teclessou et al., (2018). Our data are then, debatable. SLE is a prototype of non-organ-specific autoimmune diseases involving circulating immune complexes Maidhof and Hilar, (2012). Its pathophysiology involves both innate and adaptive immunity Pan et al., (2020). Although the signature of SLE is anti-nuclear antibodies, cytokines play a very important role in activation, differentiation and maturation of peripheral blood mononuclear cells (Lourenço and La Cava, 2009).

SLE selectively affects more women of childbearing age than men, with a sex ratio of 9 women to 1 man Ibrahim et al., (2017) In our study, All of SLE patients enrolled were women with a mean age of 32.20 years. They enrolled in their study on cytokines and effector cells in SLE, 100% of women (Méndez-Flores et al., 2016). Similarly, investigating the distribution of T and B lymphocytes in SLE in 2016, recruited SLE patients, 99% of whom were female (Szabó et al., 2016). This supports a predisposition of women to undergo SLE. Indeed, female hormones (estrogen and progesterone), through potential mechanisms, modulate the loss of immune tolerance and regulate the production of pathogenic autoantibodies in SLE Hughes and Choubey, (2014). In addition, CD40 gene, involved in the pathogenesis of SLE, is localized on the X chromosome and then X bi-allelism in women would increase their exposure to the disease Tsokos, (2011).

In our study, all SLE patients were photosensitive and were positive for anti-DNA antibodies, whereas 60% of them had nonerosive arthristis and 40% of them had discoid rash and oral/nasopharyngeal ulcers. Indeed, malar rash, discoid rash, photosensitivity, oral ulcers and nonerosive arthritis are the first five criteria of the American College of Rheumatology's (ACR), for the diagnosis of SLE and anti-DNA antibodies are important biological markers of these criteria (Eilertsen et al., 2009). In Togo, in a retrospective study from 1991 to 2003 on 16 SLE patients, they found 87.5% of discoid lupus, 56.25% of malar rash, 87.5% of polyarthralgia and 96% positivity to anti-DNA antibodies (Kombate et al., 2008). Also, in a study conducted in Benin on 33 SLE patients from 2000 to 2013, found 94.3% of articular manifestations, 70.7% of mucocutaneous manifestations and 15 cases positive for anti-DNA antibodies Zomalheto et al., (2014). These data are then consistent with clinical and biological manifestations observed in our study.

We found that the most common treatment administrated to SLE patients was Prednisone alone or combined with methotrexate or azathioprine. Other molecules used were primalan, dermoval, locoid ointment and fucidin ointment. Because SLE is an exacerbating autoimmune disease, the purpose of treatment is to modulate or suppress the immune response while preventing further infections Fava and Petri, (2019). Depending on the severity of the disease and lesions, treatments used conventionally are immunomodulators such as hydroxychloroquine, corticosteroids such as Prednisone, dihydrofolate reductase-inhibiting antimetabolites such as Methotrexate (D-penicillamine), immunosuppressants such as Azathioprine, antiallergic agents such as Mequitazine and dermocorticoids such as Clobetasol propionate and locoid ointment Walling and Sontheimer, (2009). In their study in Benin in 2014, they found that 91% of SLE patients were treated with corticosteroids Zomalheto et al., (2014). They also showed in a retrospective study in Senegal in 2017 that molecules prescribed in SLE were corticosteroids, synthetic antimalarial drugs and D-penicillamines Dioussé et al., (2017). Thus, the treatments administered to patients in this study fit well in the context of SLE.

In our study, there was a decrease in frequencies of SLE patients CD4<sup>+</sup> T cells with a defect of their activation showed by the activation marker CD69 ( $p=0.0057$ ). This result was consistent with who found low levels of T cells in Italian SLE patients Silvestris et al., (2003). Indeed, one of the biological criteria for the diagnosis of SLE according to the ACR is lymphopenia at a threshold lower than 1500/mm<sup>3</sup> observed twice and it has been proven that this lymphopenia mainly affects CD4<sup>+</sup> T cells due to their high apoptosis Martin et al., (2017). It has been also shown that in SLE, there is phenotypic and functional alteration of T cells and physiological perturbations of their TCR Crispín et al., (2013). Moreover, abnormalities in certain T cell signalling pathways leading to defects in T cell activation in SLE patients have been identified Mak and Kow, (2014). Thus, CD4<sup>+</sup> T cells lymphopenia, added to the TCR alteration and abnormalities in the T cells signaling pathways in SLE patients would explain the decrease in activation of CD4<sup>+</sup>T cells in SLE patients observed in our study.

B-cell expression was elevated in SLE patients compared to healthy donors in our study. Indeed, B lymphocytes would play an important role in autoimmune diseases through the production of a large number of anti-nuclear autoantibodies and SLE patients are believed to express recombination activating genes (RAG) aberrantly in peripheral B cells, leading to mutation of their receptor and the development of self-reactive B cells Hofmann et al., (2018). Moreover, T cell-B cell interaction is believed to result in inadequate anergic signals to self-reactive B cells and increase their survival Yap and Chan, (2019). This would explain the increase in B cells expression observed in our study.

SLE patients were shown to have an inflammatory environment with significant IL-6 production ( $p=0.0225$ ) in our study. also showed in 2006 that Egyptian SLE patients produced high levels of IL-6 Sabry et al., (2006). Similarly, they showed high IL-6 production by PBMCs from SLE patients in Mexico De la Cruz-Mosso et al., (2018). IL-6 is a member of pro-inflammatory (type I) cytokines family, which induces the expression of various proteins responsible for acute inflammation and plays an important role in the proliferation and differentiation of effector cells Uciechowski and Dempke, (2020). Thus, in a chronic inflammatory context such as SLE, high levels of IL-6 have been correlated with the progression of autoimmune disease Yao et al., (2014).

Concerning type II cytokines, SLE patients produced significantly the immuno-modulatory cytokine IL-10 compared to healthy donors. Our results are consistent with who found high levels of IL-10 in children with SLE in Brazil in 2017 Cavalcanti et al., (2017). also showed that SLE patients in Thailand produced more IL-10 than healthy individuals Rianthavorn et al., (2013). IL-10 is an anti-inflammatory cytokine first isolated from mouse Th2 lymphocytes and has inhibitory activity on T cells, monocytes, macrophages, dendritic and NK cells, MHC and pro-inflammatory cytokines (Lourenço and La Cava, 2009). In addition to its inhibitory actions, IL-10 is believed to promote B-cell-mediated functions and improve antibody production Beebe et al., (2002). Thus, increased IL-10 production in SLE patients could explain the hyperactivity of their B cells and the production of autoantibodies, two key features of immune system deregulation in SLE.

In view of the high IL-6 levels associated with high IL-10 levels obtained in the SLE patients in this study, we evaluated their inflammatory balance by calculating an IL-10/IL-6 ratio that was above 1 but remained lower than healthy donors ratio. A few studies have reported on the inflammatory balance of IL-10 and IL-1 $\beta$  between different groups of SLE patients, but provide less information on the inflammatory balance of IL-10/IL-6 in SLE compared to healthy individuals Yao et al., (2016). Indeed, IL-1 $\beta$ , IL-12, IL-17A and TNF $\alpha$  are all pro-inflammatory cytokines involved in SLE since it is a chronic inflammatory disease. But beyond these, IL-6 plays a leading role by acting alone or with other cytokines in the activation and differentiation of B cells into immunoglobulin-producing cells, as well as the proliferation and differentiation of T cells (Lourenço and La Cava, 2009). Several studies have also shown the involvement of IL-6 in various autoimmune diseases, such as SLE and rheumatoid arthritis (Uciechowski and Dempke, 2020; Gabay, 2006). Our study therefore provides an additional benefit by evaluating the IL-10/IL-6 cytokine balance in SLE patients and consolidates their inflammatory state despite the high levels of IL-10 observed in them compared to healthy donors.

## 5. CONCLUSIONS

SLE is one of the most serious systemic autoimmune diseases in the black population, particularly for women of childbearing age. The aim of our study was investigate the mononuclear blood cells and their cytokines profile in Togolese SLE patients. This study showed an impaired activation of CD4<sup>+</sup>CD69<sup>+</sup> cells in SLE patients. In addition, SLE patients were shown to have elevated serum IL-6 and IL-10 levels compared to healthy donors. IL-6 and IL-10 would play key role in the pathophysiology of SLE in Togo and could be potential markers for therapeutic follow-up of togolese SLE patients. Since the number of SLE patients in this study was small, further studies including more patients would support our data.

## Abbreviations

PBMC: Peripheral Blood Mononuclear Cells; SLE: Systemic Lupus Erythematosus; CD: Cluster of Differentiation; IL: Interleukin; RAG: Recombination-activating gene; EDTA: Ethylenediamine tetraacetic acid; DPBS: Dulbecco's Phosphate-Buffered Saline; FBS: Foetal Bovine Serum; RPMI: Roswell Park Memorial Institute; FACS: Fluorescence Activated Cell Sorting; APC: Allophycocyanin; FITC: Fluorescein isothiocyanate; PE: Phycoerythrin; ELISA: Enzyme Linked Immuno Sorbent Assay; HPR: Horseradish Peroxidase; TMB: Tetramethylbenzidin; ACR: American college of rheumatology; Ab: Antibodies; ANAs: Anti-nuclear antibodies; RNA: Ribonucleic acid; DNA: Desoxyribonuclei acid; Sm: Smith; U1-RNP: U1 particle of *ribonucleoprotein*; SSA: Sjögren's-syndrome-related antigen A; SSB: Sjögren's-syndrome-related antigen B; TGF $\beta$ : Transforming growth factor beta; IFN $\gamma$ : Interferon gamma; TCR: T cell receptor; Th: T helper cells; CBRS: Comité de Bioéthique pour la Recherche en Santé; CHU: Centre Hospitalier Universitaire.

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## Authors' contributions

GK, CNT, MK, MS and PET designed the study protocol. PET, MAO, CNT, GK and OO enrolled the patients and healthy donors, performed assays and write the manuscript. PET, MR, MOA, CNT analyzed data. BS, YA, LBM and AB validated the manuscript.

## Ethics approval and consent to participate

This study received ethical approval from the Bioethics Committee for Health Research (CBRS) of TOGO Health Ministry, under the registration number N°02/2020/CBRS. The study was explained in details to the participants in local languages (Kotokoli, Kabye, Ewe or French) and all participants gave written informed consent according to the guidelines of Declaration of Helsinki.

## Informed consent

Not applicable.

## Conflicts of interests

The authors declare that there are no conflicts of interests.

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## Data and materials availability

All data associated with this study are present in the paper.

## REFERENCES AND NOTES

1. Beebe AM, Cua DJ, de Waal Malefyt R. The role of interleukin-10 in autoimmune disease: Systemic lupus erythematosus (SLE) and multiple sclerosis (MS). *Cytokine Growth Factor Rev* 2002; 13:403-12. doi: 10.1016/s1359-6101(02)00025-4
2. Cavalcanti A, Santos R, Mesquita Z, Duarte AL, Lucena-Silva N. Cytokine profile in childhood-onset systemic lupus erythematosus: A cross-sectional and longitudinal study. *Braz J Med Biol Res* 2017; 50:e5738. doi: 10.1590/1414-431x20175738
3. Crispín JC, Hedrich CM, Tsokos GC. Gene-function studies in systemic lupus erythematosus. *Nat Rev Rheumatol* 2013; 9:47 6-84. doi: 10.1038/nrrheum.2013.78
4. Dar SA, Das S, Ramachandran VG, Bhattacharya SN, Mustafa MD, Banerjee BD, Verma P. Alterations in T-lymphocyte sub-set profiles and cytokine secretion by PBMC of systemic lupus erythematosus patients upon in vitro exposure to organochlorine pesticides. *J Immunotoxicol* 2012; 9:85-95. doi: 10.3109/1547691x.2011.642103
5. De la Cruz-Mosso U, García-Iglesias T, Bucala R, Estrada-García I, González-López L, Cerpa-Cruz S, Parra-Rojas I, Gámez-Nava JI, Pérez-Guerrero EE, Muñoz-Valle JF. MIF promotes a differential Th1/Th2/Th17 inflammatory

- response in human primary cell cultures: Predominance of Th17 cytokine profile in PBMC from healthy subjects and increase of IL-6 and TNF- $\alpha$  in PBMC from active SLE patients. *Cell Immunol* 2018; 324:42-9. doi: 10.1016/j.cellimm.2017.12.010
6. Dioussé PBA, Dione H, Ps T, Bammo M, Seck F, Gueye N, Diop MM, Faye AF, Dieng MT, Diop BM, Mourtalla KAM. Profil épidémiologique des maladies auto-immunes systémiques dans un service de Dermatologie. *Rev Afr Méd Interne* 2017; 4.
7. Eilertsen G, Becker-Merok A, Nossent JC. The influence of the 1997 updated classification criteria for systemic lupus erythematosus: Epidemiology, disease presentation and patient management. *J Rheumatol* 2009; 36:552-9. doi: 10.3899 /jrheum.080574
8. Fava A, Petri M. Systemic lupus erythematosus: Diagnosis and clinical management. *J Autoimmun* 2019; 96:1-13. doi: 10.1016/j.jaut.2018.11.001
9. Gabay C. Interleukin-6 and chronic inflammation. *Arthritis Res Ther* 2006; 8(Suppl 2):S3. doi: 10.1186/ar1917
10. Hofmann K, Clauser AK, Manz RA. Targeting B Cells and Plasma Cells in Autoimmune Diseases. *Front Immunol* 2018; 9 :835. doi: 10.3389/fimmu.2018.00835
11. Hughes GC, Choubey D. Modulation of autoimmune rheumatic diseases by oestrogen and progesterone. *Nat Rev Rheumatol* 2014; 10:740-51. doi: 10.1038/nrrheum.2014.144
12. Ibrahim IN, Mamman AI, Adaji SE, Hassan A, Babadoko AA. Prevalence of lupus anticoagulant in women with spontaneous abortion in Zaria. *Niger J Clin Pract* 2017; 20:11 45-9. doi: 10.4103/njcp.njcp\_125\_16
13. Kombate K, Saka B, Oniankitan OI, Sodonougbo P, Mouhari-Toure A, Tchangai-Walla K, Pitche P. (Systemic lupus erythematosus in Lomé, Togo). *Med Trop (Mars)* 2008; 68:283-6.
14. Lourenço EV, La Cava A. Cytokines in systemic lupus erythematosus. *Curr Mol Med* 2009; 9:242-54. doi: 10.2174/156652409787847263
15. Maidhof W, Hilas O. Lupus: An overview of the disease and management options. *P t* 2012; 37:240-9.
16. Mak A, Kow NY. The pathology of T cells in systemic lupus erythematosus. *J Immunol Res* 2014; 2014:419029. doi: 10.1155 /2014/419029
17. Martin M, Guffroy A, Argemi X, Martin T. (Systemic lupus erythematosus and lymphopenia: Clinical and pathophysiological features). *Rev Med Interne* 2017; 38:603-13. doi: 10.1016/j.revmed.2017.01.005
18. Méndez-Flores S, Hernández-Molina G, Enríquez AB, Faz-Muñoz D, Esquivel Y, Pacheco-Molina C, Furuzawa-Carballeda J. Cytokines and Effector/Regulatory Cells Characterization in the Physiopathology of Cutaneous Lupus Erythematosus: A Cross-Sectional Study. *Mediators Inflamm* 2016; 2016:7074829. doi: 10.1155/2016/7074829
19. Möckel T, Basta F, Weinmann-Menke J, Schwarting A. B cell activating factor (BAFF): Structure, functions, autoimmunity and clinical implications in Systemic Lupus Erythematosus (SLE). *Autoimmun Rev* 2021; 20:102736. doi: 10.1016/j.autrev.2020.102736
20. Mortezaagholi S, Babaloo Z, Rahimzadeh P, Ghaedi M, Namdari H, Assar S, Azimi Mohamadabadi M, Salehi E. Evaluation of PBMC Distribution and TLR9 Expression in Patients with Systemic Lupus Erythematosus. *Iran J Allergy Asthma Immunol* 2016; 15:229-36.
21. Ocampo-Piraquive V, Nieto-Aristizábal I, Cañas CA, Tobón GJ. Mortality in systemic lupus erythematosus: Causes, predictors and interventions. *Expert Rev Clin Immunol* 2018; 14:1043-53. doi: 10.1080/1744666x.2018.1538789
22. Pan L, Lu MP, Wang JH, Xu M, Yang SR. Immunological pathogenesis and treatment of systemic lupus erythematosus. *World J Pediatr* 2020; 16:19-30. doi: 10.1007/s12519-019-00229-3
23. Ramírez Sepúlveda JI, Bolin K, Mofors J, Leonard D, Svenungsson E, Jönsen A, Bengtsson C, Nordmark G, Rantapää Dahlqvist S, Bengtsson AA, Rönnblom L, Sjöwall C, Gunnarsson I, Wahren-Herlenius M. Sex differences in clinical presentation of systemic lupus erythematosus. *Biol Sex Differ* 2019; 10:60. doi: 10.1186/s13293-019-0274-2
24. Raphael I, Nalawade S, Eagar TN, Forsthuber TG. T cell subsets and their signature cytokines in autoimmune and inflammatory diseases. *Cytokine* 2015; 74:5-17. doi: 10.1016/j.cyt.2014.09.011
25. Rianthavorn P, Chokedeemeeboon C, Deekajorndech T, Suphapeetiporn K. Interleukin-10 promoter polymorphisms and expression in Thai children with juvenile systemic lupus erythematosus. *Lupus* 2013; 22:721-6. doi: 10.1177/0961203313486192
26. Sabry A, Sheashaa H, El-Husseini A, Mahmoud K, Eldahshan KF, George SK, Abdel-Khalek E, El-Shafey EM, Abo-Zenah H. Proinflammatory cytokines (TNF-alpha and IL-6) in Egyptian patients with SLE: Its correlation with disease activity. *Cytokine* 2006; 35:148-53. doi: 10.1016/j.cyt.2006.07.023
27. Silvestris F, Grinello D, Tucci M, Cafforio P, Dammacco F. Enhancement of T cell apoptosis correlates with increased serum levels of soluble Fas (CD95/Apo-1) in active lupus. *Lupus* 2003; 12:8-14. doi: 10.1191/0961203303lu250oa
28. Szabó K, Papp G, Szántó A, Tarr T, Zeher M. A comprehensive investigation on the distribution of

- circulating follicular T helper cells and B cell subsets in primary Sjögren's syndrome and systemic lupus erythematosus. *Clin Exp Immunol* 2016; 183:76-89. doi: 10.1111/cei.12703
29. Teclessou JN, Saka B, Akakpo SA. Connective tissue diseases in the hospital setting in Lomé: a retrospective study of 231 cases. *Pan Afr Med J* 2018; 30:176. doi: 10.11604/2Fpamj.2018.30.176.14565
30. Tsokos GC. Systemic lupus erythematosus. *N Engl J Med* 2011; 365:2110-21. doi: 10.1056/nejmra1100359
31. Uciechowski P, Dempke WCM. Interleukin-6: A Master player in the Cytokine Network. *Oncology* 2020; 98:131-7. doi: 10.1159/000505099
32. Walling HW, Sontheimer RD. Cutaneous lupus erythematosus: Issues in diagnosis and treatment. *Am J Clin Dermatol* 2009; 10:365-81. doi: 10.2165/11310780-000000000-00000
33. Yao X, Huang J, Zhong H, Shen N, Faggioni R, Fung M, Yao Y, Shen N, Faggioni R, Fung M, Yao Y. Targeting interleukin-6 in inflammatory autoimmune diseases and cancers. *Pharmacol Ther* 2014; 141:125-39. doi: 10.1016/j.pharmthera.2013.09.004
34. Yao Y, Wang JB, Xin MM, et al. Balance between inflammatory and regulatory cytokines in systemic lupus erythematosus. *Genet Mol Res* 2016; 15. doi: 10.4238/gmr.15027626
35. Yap DYH, Chan TM. B Cell Abnormalities in Systemic Lupus Erythematosus and Lupus Nephritis-Role in Pathogenesis and Effect of Immunosuppressive Treatments. *Int J Mol Sci* 2019; 20. doi: 10.3390/ijms20246231
36. Zomaheto Z, Assogba M, Agbodande A, Atadokpede F, Gounongbe M, Avimadje M. Pattern of systemic lupus erythematosus in Benin and West African patients. *Tunis Med* 2014; 92:707-10.